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EFFECTS OF PHASE DIFFERENCE ON THE H-ANTIGEN TRANSDUCTION IN SALMONELLA DIPHASIC STRAINS.

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In order to make clear the relationship of two loci (H_1 and H_2) to the H-antigen phase variation, effects of phase difference on the H-antigen transduction in Salmonella diphasic strains were observed.

Materials and Methods.

Three Salmonella diphasic strains, Sal. abony SW-803 (Fla$^+$, b:enx), Sal. heidelberg SW-1092 (Fla$^-$, r:1,2) and Sal. typhimurium TM-2 (Fla$^+$, i:1,2), were used in this experiment.

~~Preparation of~~ The transducing lysate from each phase of these strains was ~~made~~ prepared as follows. Phase 1 and phase 2 colonies were picked up from the EMB-lactose plate cultures of the diphasic strains, and were inoculated into penassay broth. After 10 hours incubation, 2 drops of each culture was inoculated into 10 ml of new penassay broth cultures with 1 small loopful (ca. 0.002 ml) of lysate as evocator of phage. At the same time cultures without lysate were prepared as controls. They were incubated 2 hours, and put into the 60°C water bath for 45 minutes to kill the bacterial cells. After that, they were centrifuged and the supernates were shaken with the chloroform and kept in refrigerator. Controls were not killed by heat but were streaked on EMB-lactose plates and the homogeneity of phase in each of the cultures was confirmed by testing ~~the test of~~ the antigen types of about 20 colonies in each.

Fla$^-$ Recipient cell cultures of each phase were prepared as follows. The culture of mixed phase was streaked on EMB-lactose plate. 10 colonies were picked up from it into penassay culture, and were kept in refrigerator after 6 hours of culture. 0.5 ml of each culture was mixed with an equal volume of lysate obtained from Fla$^+$-

strains and brushed on motility-gelatine-agar media (MGA), and the antigen types of the developed swarms were tested. Homogeneous cultures for phase 1 or phase 2 were checked from them, and original stored cultures of them were used as recipient cell cultures.

For transduction experiments, lysate was mixed with penassay broth recipient cell culture in equal volume, and brushed on MGA (in case of Fla^+-x Fla^-) or MGA supplemented with antiserum for the antigens of recipient cell (in case of Fla^+-x Fla^+). The swarms developed as a result of transduction were picked up onto EMB-lactose agar media and their antigen types were tested by slide agglutination ~~test~~. The antigen type of alternative phase was tested with ~~the~~ successive penassay subcultures of colonies or, when it is still monophasic, after selecting by ~~the~~ MGA containing antiserum for the expressed antigen.

Experimental Results.

The first experiment was done by treating the lysate of each phase of Sal. abony (SW-803, Fla^+, b:enx) to the cells of each phase of Sal. heidelberg (SW-1092, Fla^-, r:1,2). Fla^+ was used as selective marker. The results obtained were summarized in Table 1.

The phase of recipient cell after transduction is just same as the phase of that before transduction. The few exceptions when phase 2 was recipient may be attributed to the contamination of phase 1 cell by the phase variation in the culture of phase 2. The transduced type of phase 1 appeared in every combinations by the frequencies from 51 to 86 %, on the other hand the transduced type of phase 2 did not appear in any combinations. These results indicate that:

(1). Phase 1 (H_1) is transduced linked to "Fla" regardless the phases of donor and recipient.

(2). Phase 2 (H_2) is not transduced linked to "Fla".

(3). The phase of transduced cells are exclusively determined by the phase of recipient cell.

From (1) and (3), it is deduced further that, in the cell which shows phase 2, H_1 is exist but is inhibited its action by some factor. On the mechanism of inhibition, two alternative hypothesis are presented.

I). ~~The~~ phase is determined by the cytoplasmic condition or some factor involved in cytoplasm.

II). H_2 suppresses the action of H_1, and H_1 is expressed only when H_2 mutates to inactive allele (h_2). Accordingly phase 1 has H_1h_2, whereas phase 2 has H_1H_2, and phase variation is caused by the following mutational change: $H_2 \rightleftarrows h_2$.

From the result obtained in this experiment, it is impossible to decide which of these alternatives represents the real phenomena, because H_2-transduced type cannot be screened in this experiment. The difference of two alternative phenomena may become clear when diphasic culture is transduced by the lysate of each phase and selected by the antiserum for H_1 and H_2antigen of recipient strain. In such experiment both H_1- and H_2-transduced types are screened, and if I) is correct, transduced cells show either phase 1 or phase 2 when transduced by phase 1 lysate as well as by phase 2 lysate. On the contraty, if hypothesis II) is correct, transduced cells show only phase 1 when transduced by phase 1 lysate,and both phase 1 and phase 2 by phase 2 lysate. On these expectations, the cells of Sal. abony (b:enx) was transduced by the lysate of each phase of Sal. typhimurium TM-2 (i:1,2), and transduced types were screened by the MGA containing anti-i and anti-enx. The result is shown in Table 2.

As can be seen in the table, differential effect of donor phase is very clear. In case of transduction by the lysate of phase 1, only phase 1 antigen is transduced, whereas in case of phase 2, both phase 1 and phase 2 were transduced. This result indicate the 2nd hypothesis II) is preferable to explain the phenomena.

According to the hypothesis II), the ratio of phase 1-transduced type and phase 2-transduced type in the experiment (phase 2 -x mixed phase) is

phase 1 : phase 2 = p : 1

(p indicates the rate of phase 1 cells in recipient culture.)

if efficiencies of transduction do not differ between H_1 and H_2. The recipient cell culture used in this experiment has contained 23 phase 1 cells in 50 tested, that is, p=0.46. Consequently, the frequencies of phase 1-transduction observed* is rather higher than the expected value from hypothesis II). The cause of this difference is not clear at present. One possible explanation is to suppose the difference of transducing efficiencies between H_1 and H_2.

(* The observed ratio is phase 1 : phase 2 = 1 : 1)

<u>Discussion.</u>

Genetic approach to the mechanism of phase variation was first made by Dr. Stocker (1949). He concluded, from the observation of cells of each phase in the cultures of diphasic strain during the growth, that phase variation is resulted from mutation and back-mutation of one locus. Lately, transduction experiment was applied to the genetic analysis of this phenomena (Lederberg and Edwards, 1953 and Lederberg, 1954), and was found out that two phases were transduced separately. This result lead to the assumption that two alternative phases are controlled by two distinct loci, H_1 and H_2 each. Present experiment confirms the result obtained previously by the transduction experiment, and more-over makes clear the relation between the Stocker's result and the result obtained ~~previously~~ by the transduction experiment, showing the leadership of H_2 locus for phase variation by suppression for H_1 and by mutation $H_2 \rightleftarrows h_2$.

The hypothesis of H_2-leadership also serves to explain the phenomena of H-antigen transduction in monophasic strain reported previously. According to the present hypothesis there are 5 possibilities of monophasic condition, that is: stabilization of H_2 (type-1), or h_2 (type-2) (designated H2 and h2 correspondingly), mutation of H_1 to inactive allele h_1

do. H_2 ...

(type-3), and loss of H_1-locus (type-4) or H_2-locus (type-5). These types are distinguishable by the transduction ~~of~~ from diphasic strain to them, as shown in Table 3. The results obtained in the previous experiment on the transduction of monophasic strains are explained well providing one of these genotypes to each of monophasic strains. For example, the behavior of Sal. typhi H-901(d:-) and Sal. paratyphi B SW-666 (b:-), to which only phase 1 is transduced from diphasic strain and give phase 1-monophasictype, are explained as they have type-5 constitution (H_1-). The behavior of Sal. paratyphi B CDC-157, which is monophasic (1,2:-) but gives diphasic strain by the transduction of H_2 from diphasic strain as well as phase 1-transduced monophasic strain, are explained as it has type-2 constitution ($H_1\underline{h_2}$). Samely, apparently peculiar changes from monophasic to diphasic in Sal. paratyphi B SW-960 (-:1,2) by the transduction of H_1 become clear assuming that the strain has type-3 constitution (h_1H_2), and the ability of phase 2-monophasic strain of Sal. abortus-equi CDC-26 to transduce H_1 to other strain is to its type-1 constitution ($H_1\underline{H_2}$). The remained one type, type-4 ($-H_2$), can be used to explain the behavior of phase 2-monophasic strain Sal. paratyphi B SW-959, to which only phase 2 is transduced from diphasic strains. Thus all of the 5 monophasic types, expected from present hypothesis, are found out in the strains studied since, and in turn monophasic strain which cannot be included in these types has not been observed yet.

The type specifisity of each phase is not altered by ~~the~~ phase variation, that is; the change of H_2 to h_2 follows the change of the component which controls the suppressive action for H_1 ~~action~~ (accordingly controls the phase variation) but does not follow the change of the component which controls the specificity of antigen type, described as follows: $H_2^{1,2} \nRightarrow h_2^{1,2}$. Whether these two parts are regarded as different loci or the part of one gene are difficult to define, yet transduction analysis gives no proof to distinguish them as different loci and the functions of these two parts are over all complemental, just as complex enzyme can be active only when apo-enzyme and co-enzyme put together, so it may be convenient at present to regard them as a genetic unit as a whole.

Table 1.

Transductions between monophasic cultures of diphasic strains.

Sal. abony (Fla$^+$, b:enx) -x Sal. heidelberg (Fla$^-$, r:1,2).

Phase of donor	Phase of recipient	Antigentypes of Fla$^+$-transduced cells						Total	Ratio of linked transduction
		Unlinked type			Linked type				
		r:(1,2)	(r):1,2	Total	b:(1,2)	(b):1,2	Total		
1 (b)	1 (r)	21	0	21	22	0	22	43	0.51
1 (b)	2 (1,2)	0	7	7	1	42	43	50	0.86
2 (enx)	1 (r)	11	0	11	30	0	30	41	0.73
2 (enx)	2 (1,2)	1	10	11	1	38	39	50	0.78
Total		33	17	50	54	80	134	184	0.73

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Table 2.

Transductions of H_1 and H_2 from monophasic cultures to diphasic culture. Sal. typhimurium TM-2 (Fla$^+$, i:1,2) -x Sal. abony SW-803 (Fla$^+$, ~~b:1,2~~). b: enx ✓

Donor	Recipient	No. of transduced cell		
		i:(enx)	(b):1,2	Total
TM-2 phase 1 (i)	SW-803 (b:enx)	19	0	19
TM-2 phase 2 (1,2)	" "	14	14	28

Table 3.

Constitutions of H_1 and H_2 locus in monophasic strains

No. (type)	H-constitution	Phase	Type given by transduction of diphasic strain ($H_1'H_2'$)	
1	H_1 $\underline{H}_2$	2	H_1' $\underline{H}_2$, H_1 H_2'	H_2-t.d.ed diphasic
2	H_1 $\underline{h}_2$	1	H_1' $\underline{h}_2$, H_1 H_2' <	H_1-t.d.ed monophasic and H_2-t.d.ed diphasic
3A 3	h_1 H_2	2	H_1' H_2 ; h_1 H_2' <	H_1-t.d.ed diphasic and H_2-t.d.ed monophasic
4	- H_2	2	H_2'	H_2-t.d.ed monophasic
5	H_1 -	1	H_1'	H_1-t.d.ed monophasic

t.d.ed=transduced